PCT/GB 99/ 03936
25 NOVEMBER 1999

INVESTOR IN PEOPLE

9918530867

GB99/3936

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT

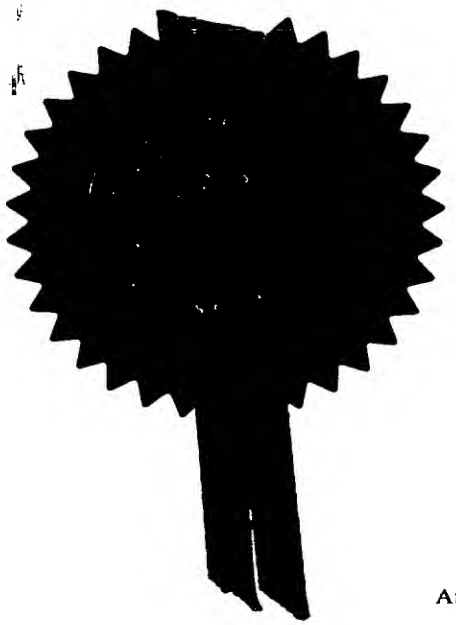
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

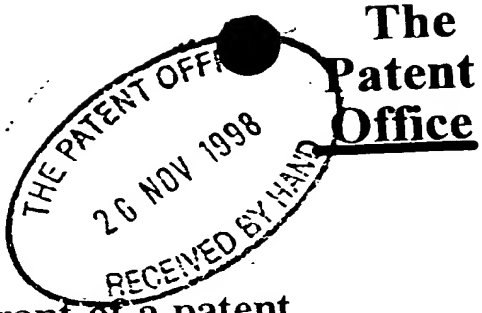
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed Andrew Gerry

Dated 8 December 1999

Patents Form 1/77



The Patent Office

26 NOV 1998

27NOV98 E407315-3 002136
P01/7700 0.00 - 9825948.4

Patents Act 1977
(Rule 16)

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference SC/N7614

2. Patent application number *(The Patent Office will fill this part)* **9825948.4**

3. Full name , address and postcode of the or of each applicant *(underline all surnames)*

KING'S COLLEGE, UNIVERSITY OF LONDON
The Strand
London
WC2R 2LS

Patents ADP number *(if you know it)* 68669330001

If the applicant is a corporate body, give the country/state of its incorporation U.K.

4. Title of the invention DIAGNOSIS OF SPONGIFORM DISEASE

5. Name of your agent *(if you have one)* Williams, Powell & Associates

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

4 St. Paul's Churchyard
London
EC4M 8AY

Patents ADP number *(if you know it)* 5830310001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earleir UK application, give the number and the filing date of the earlier application.

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(answer 'Yes if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Diagnosis of Spongiform Disease

This invention relates to the diagnosis of spongiform disease, especially bovine spongiform encephalomyelitis (BSE).

In our co-pending application WO/9702667 we have disclosed a new diagnostic test for spongiform encephalopathy and other de-myelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and other animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, the new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. Foremost among the bacteria that are involved in the induction of the auto-immune reaction are Acinetobacter species, especially Acinetoabacter calcoaceticus. The diagnostic test based on the new model opens up the possibility of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further auto-immune attack on the animal's own myelin.

In our co-pending UK patent application 9805913.2, we have confirmed the presence of elevated levels of Acinetobacter IgA antibodies in sera of patients suffering from multiple sclerosis and we have postulated that this observation will be also confirmed in sera of patients with CJD.

In further tests we have now confirmed the presence of antibodies to bovine myelin and also to bovine myelin neurofilaments in the sera of cows that have died from BSE. These antibodies are of the IgA type. These findings confirm the validity of the model described above and permit the conclusion that we have discovered a general pattern of the origin of similar diseases that occur or may occur in vertebrates including humans and other farm animals e.g. in poultry farms. Our latest results also provide the basis of a further test for the early identification of these diseases, especially incipient BSE in cows. This further test may either be alternative to or additional to that based on the detection of IgA antibodies to Acinetobacter species e.g. Acinetobacter calcoaceticus.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with Acinetobacter is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. It is possible that the nasal passages are the site of infection, resulting from inhalation of dust formed from dried sewage or animal excrement and carrying Acinetobacter. The knowledge of this mechanism implies the need for improved hygiene practices in the rearing of farm animals.

Experimental

Assays for the above mentioned organisms are described in our co-pending applications identified above, the contents of which are hereby incorporated by reference. Similar assay procedures using myelin protein or neurofilaments thereof as test antigens are described below.

ELISA TEST:

(1) Aliquots of 200ul of the antigen suspension A or B were absorbed on 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4 deg. Cent. (Antigen A is bovine myelin from Sigma at a concentration of 5ug/ml and antigen B is bovine neurofilaments from Sigma also at a concentration of 5ug/ml).

(2) The plates are then washed 3 times with phosphate buffered saline (PBS) 0.1% (v/v) Tween 20.

(3) Aliquots of 300ul of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween) in PBS is added to each well and incubated for one hour at 37 deg. Cent.

(4) The plates are then washed 3 times with PBS. Tween 20.

(5) Aliquots of 200ul serum samples (test or control) diluted 1/200 in PBS. Tween is added and incubated for 2 hours at 37 deg. Cent.

(6) The plates are then washed 3 times with PBS. Tween 20.

(7) Aliquots of 200ul of peroxidase conjugated rabbit anti-cow IgA (alpha chain) diluted 1/4000 with PBS. Tween are added and incubated for 2 hours at 37 deg. Cent.

(8) The plates are then washed 3 times with PBS. Tween 20.

(9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200ul per well of 0.5 mg/ml (2,2'-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/ phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

(10) The reaction is then stopped with 100ul of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

(11) All assays are done under coded conditions, in that the tester is unaware of the origin of the serum being studied (Test or control).

(12) All tests are done in duplicate.

RELEVANCE OF ASSAY

This assay is a novel way of diagnosing cattle suffering from "bovine spongiform encephalopathy" (BSE) in that it describes a test where antibodies to 2 brain antigens can be determined in the BSE sera.

Any reading in excess of 2 standard deviations of the healthy controls would indicate a positive response.

Furthermore the test should be positive (above 2 standard deviations) for both antigens: (A) Bovine myelin protein and (B) Bovine neurofilaments.

This assay is an advance on diagnosis of BSE in cattle and has not been previously described.

This is the first assay that describes measurements of autoantibodies to brain antigens in BSE affected cattle.

Results are shown in the attached Figures 1 and 2.

From the foregoing it will be appreciated that the present invention comprises:

A method for diagnosing spongiform disease in vertebrates which comprises assaying a biological sample for IgA antibodies to vertebrate myelin or myelin neurofilaments

A method as defined above in which the vertebrate is bovine.

A method as defined above in which the vertebrate is human.

A method as defined above in which a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

A diagnostic kit for the detection of spongiform disease in vertebrates comprising, as test antigen, vertebrate myelin or myelin neurofilaments.

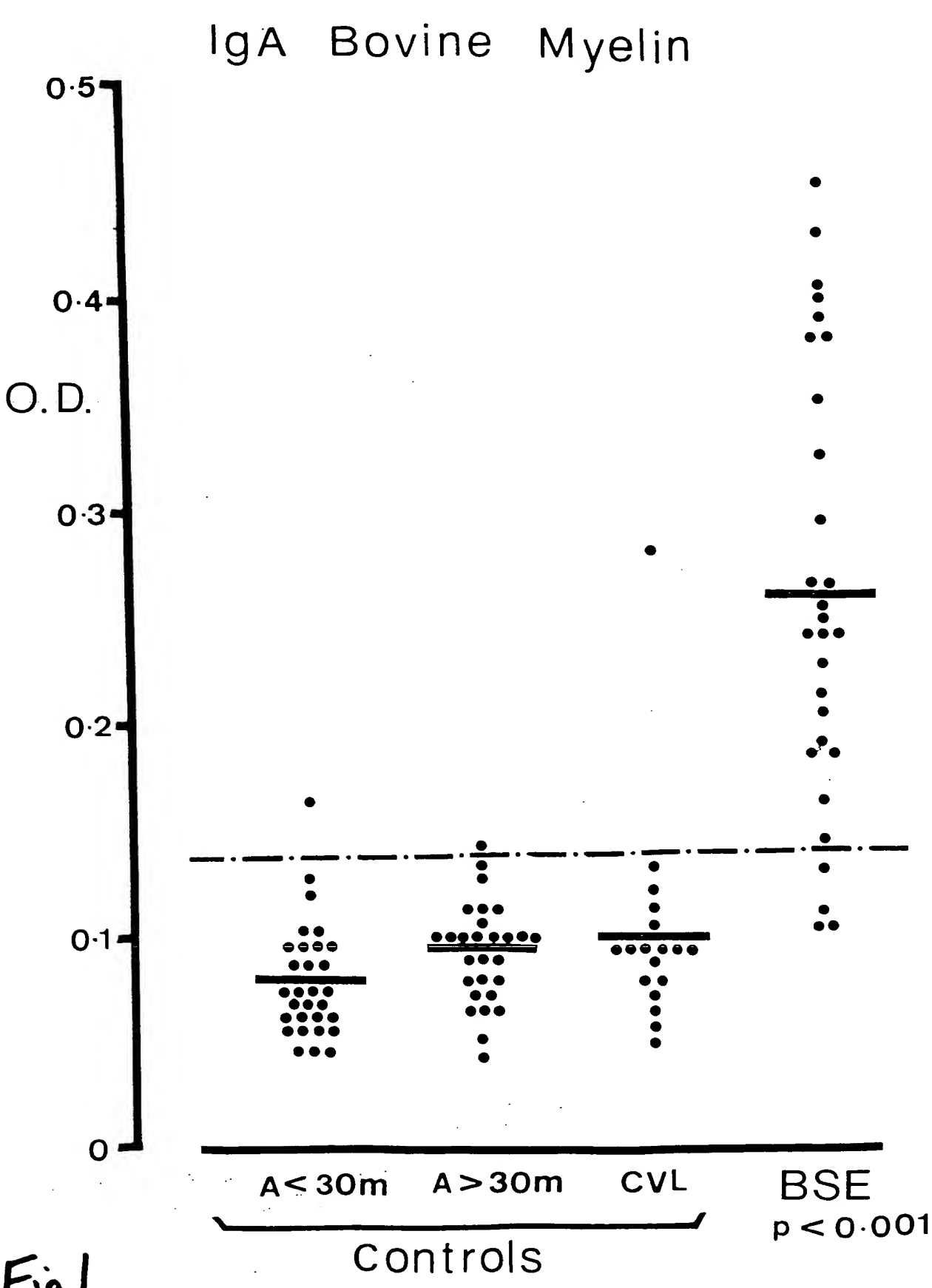

IgA Bovine Myelin
0·5
0·4
O.D.
0·3
0·2
0·1
0
A< 30m
A>30m
CVL
BSE
p < 0·001
Controls

Fig 1

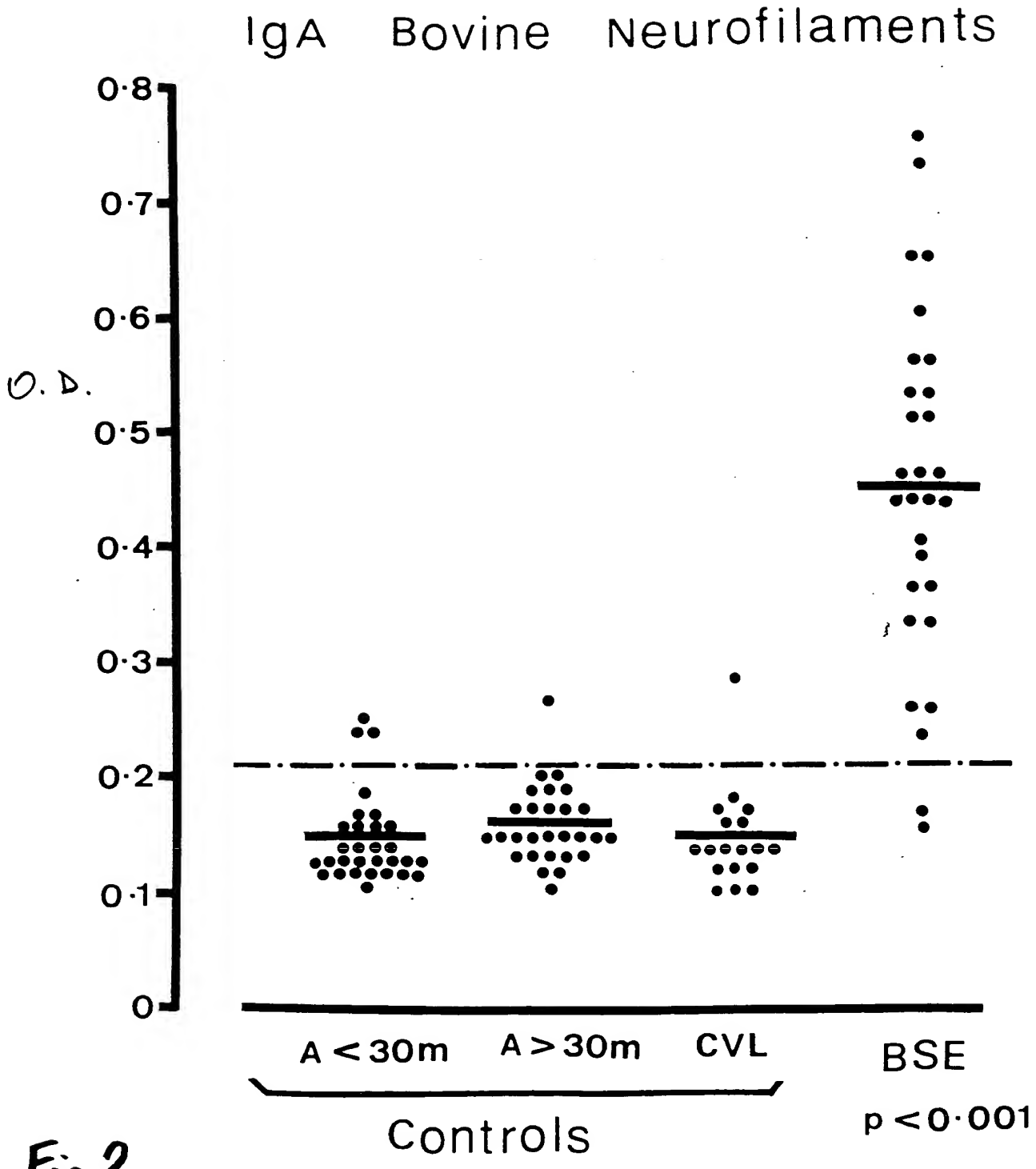

IgA Bovine Neurofilaments
0·8
0·7
0·6
O. D.
0·5
0·4
0·3
0·2
0·1
0
A < 30m
A > 30m
CVL
BSE
Controls
p < 0·001

Fig 2